REMARKS

Claims 49–61 are pending in this Application. The Applicant has cancelled claims 49, 54 and 55 without prejudice to his rights to pursue the subject matter of these claims in this or other applications. The Applicant has added new claims 58–61 which more clearly define the claimed subject matter and properly fall within the subject matter of the elected claims. Support for the newly added claims is found throughout the specification and/or in the claims as originally filed. No new matter has been entered.

New independent claim 58 merely claims a method of classifying gene expression in a test subject relative to a population of control subjects that includes subjects having osteoarthritis and healthy subjects. New claim 58 comprises a step of quantifying a level of RNA encoded by a netrin 4 (NTN4) gene in a blood sample from the test subject, and a subsequent step of comparing the level in the sample from the test subject with levels of RNA encoded by the gene in blood samples from the subjects having osteoarthritis and in blood samples from the healthy subjects. The new claim concludes that a determination that the level in the sample from the test subject is statistically similar to the levels in the samples from the subjects having osteoarthritis and is statistically different from the levels in the samples from the healthy subjects classifies the level in the sample from the test subject with the levels from the samples from the subjects having osteoarthritis; and/or concludes that a determination that the level in the sample from the test subject is statistically different from the levels in the samples from the subjects having osteoarthritis and is statistically similar to the levels in the samples from the healthy subjects classifies the level in the sample from the test subject with the levels in the samples from the healthy subjects. Support for reciting comparison of biomarker RNA levels of a test subject with those of control subjects having a disease (i.e. osteoarthritis) and with those of healthy control subjects, and determination of a statistically significant similarity or difference therebetween can be found in the published application US 2005/0123938 (hereinafter "Published Application"), for example at paragraph [0128] ("*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different*

levels) were true"), at paragraph [0129] ("*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"). Support for reciting classification of a test subject level with specific control levels can be found, for example, at claim 12 as originally filed ("*d) determining whether the level of said one or more gene transcripts of step a) classify with the levels of said transcripts in step b) as compared with the levels of said transcripts in step c)*"), at paragraph [0136] (relating to "*Methods that can be used for class prediction analysis*"), [0398] ("*Blood samples were taken from patients who were diagnosed with osteoarthritis and a specific stage of osteoarthritis as defined herein. Gene expression profiles were then analysed and compared to profiles from patients unaffected by any disease.*") and paragraph [0400], describing the osteoarthritis versus healthy control clustering data shown in Figure 22.

New claim 59 has been added, limiting the control subjects having osteoarthritis to those which are not subject to systemic steroids and which do not have any one of hypertension, obesity, allergies, mild osteoarthritis, or severe osteoarthritis.

New claims 60 and 61 have been added, limiting the control subjects having osteoarthritis to those which have moderate osteoarthritis or marked osteoarthritis, respectively.

Claim 51 has been amended in order to clarify that said levels of RNA encoded by said gene are in blood samples leukocytes which include all of the types of leukocytes in whole blood, i.e. of blood samples which include granulocytes in addition to mononuclear cells (T-lymphocytes, B-lymphocytes and monocytes). This phrase finds clear support in the specification, including at Figure 5C which shows standardized levels of insulin gene in each of the fractions of leukocytes which collectively constitute unfractionated leukocytes, i.e. granulocytes, T-lymphocytes, B-lymphocytes and monocytes (labeled "G.R.", "CD 3+", "CD19" and "MONO", i.e., respectively). It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes (refer, for example, to the enclosed Abstract of Casey *et al.*, 1988. simplified plastic embedding and immunohistologic

technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). The fact that granulocytes (G.R.), lymphocytes [T-lymphocytes (CD 3+) and B-lymphocytes (CD19+)] and monocytes (MONO) represent all of the types of leukocytes found in blood is taught at Fig. A.23 Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. (attached) which clearly teaches that leukocytes are composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. Additional support for the term "leukocytes" is found at paragraphs [0004] and [0089] of the Published Application.

Specification

The Examiner has objected to Applicant's amendment received 7/27/04 on the grounds that the incorporation by reference of 10/601,518 constitutes new matter. In order to address the Examiner's concern Applicant has amended the specification as described above so as to no longer claim incorporation by reference of 10/601,518.

35 U.S.C. § 112, 2nd Paragraph, Rejections – Indefiniteness

Claim 51 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which the Applicant regards as the invention. More particularly, the phrase "unfractionated samples of lysed blood" has been objected to.

Applicant respectfully traverses the rejection, including as it would apply to any of the newly added and/or amended claims. Applicant submits that the many embodiments of blood samples disclosed in the specification do not render the referenced phrase indefinite. However, solely for the purposes of expediting prosecution, Applicant has now amended claim 51 so as to limit the blood samples to those which comprise "*leukocytes which have not been fractionated into cell types*". Applicant respectfully submits that the limitation of a blood sample which "**comprises leukocytes which** have not been fractionated into cell types" finds clear support in the specification. As noted above, support is found throughout the specification including at Figure 5C, which shows

the fractionation of different types of leukocytes which collectively constitute unfractionated leukocytes, i.e. granulocytes, T-lymphocytes, B-lymphocytes and monocytes (labeled "G.R.", "CD 3+", "CD19" and "MONO", i.e., respectively). It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes (refer, for example, to the enclosed Abstract of Casey *et al.*, 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). The fact that granulocytes, lymphocytes (T-lymphocytes and B-lymphocytes) and monocytes represent all of the types of leukocytes found in blood is evident at Fig. A.23 of Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. at, (enclosed), which clearly teaches that leukocytes are composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. The ordinarily skilled artisan will readily understand, particularly in view of the legend of Figure 5C which recites "*FIG. 5C shows... each fractionated cell from whole blood.*", that data representing each type of blood leukocyte is represented in Figure 5C; that "G.R." is an acronym representing granulocytes; that "CD 3+" refers to CD3+ cells, i.e. T-lymphocytes; that "CD19" refers to CD19+ cells, i.e. B-lymphocytes; that "MONO" is an acronym representing monocytes; and that all lymphocytes are represented in Figure 5C since whole lymphocytes consist of T- and B-lymphocytes. Literal support for the term "leukocytes" can be found at paragraphs [0005] and [0089] of the published application.

In view of this amendment and remarks clarifying the claimed embodiments, Applicant respectively requests that these rejections be reconsidered and withdrawn.

35 U.S.C. § 112, 1st Paragraph Rejections, - Written Description

Claims 51–57 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

The office action states that the limitation "unfractionated samples of lysed blood" appears to be new matter. Applicant traverses the rejection, but has now removed the referenced phrase from the claims, solely for the purposes of advancing prosecution.

As described above in the response to the 35 U.S.C. § 112, 2nd paragraph rejections, Applicant has now amended claim 51 which, for greater clarity, limits each claimed blood sample to one which "*comprises leukocytes which have not been fractionated into cell types*", which finds clear written support in the specification, as described above.

In view of this amendment and remarks, Applicant respectively requests that this rejection be reconsidered and withdrawn.

35 U.S.C. § 112, 1st Paragraph Rejections, - Enablement

Claims 49-57 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. Applicant respectfully traverses the rejections.

Nature of the Invention and Scope of claims

The Examiner contends that the claims are extremely broad because they set forth that any or all comparison between a test subject and RNA level from "control subjects" is indicative of disease. The Examiner further contends that the claims are broad with regard to control subjects which could encompass patients with various conditions.

In the interest of expediting prosecution of the instant application, Applicant has now cancelled independent claim 49 and has added new independent claim 58, from which all other claims depend, which merely claims a method of classifying gene expression in a test subject relative to a population of control subjects that includes subjects having osteoarthritis and healthy subjects. New claim 58 comprises a step of quantifying a level of RNA encoded by a netrin 4 (NTN4) gene in a blood sample from the test subject, and a subsequent step of comparing the level in the sample from the test subject with levels of RNA encoded by the gene in blood samples from the subjects having osteoarthritis and in blood samples from the healthy subjects. The new claim concludes that a determination that the level in the sample from the test subject is statistically similar to the levels in the samples from the subjects having osteoarthritis and is statistically different from the levels in the samples from the healthy subjects classifies

the level in the sample from the test subject with the levels from the samples from the subjects having osteoarthritis; and/or concludes that a determination that the level in the sample from the test subject is statistically different from the levels in the samples from the subjects having osteoarthritis and is statistically similar to the levels in the samples from the healthy subjects classifies the level in the sample from the test subject with the levels in the samples from the healthy subjects. Support for reciting comparison of biomarker RNA levels of a test subject with those of control subjects having a disease (i.e. osteoarthritis) and with those of healthy control subjects, and determination of a statistically significant similarity or difference therebetween can be found in the published application US 2005/0123938 (hereinafter "Published Application"), for example at paragraph [0128] ("*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"), at paragraph [0129] ("*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"). Support for reciting classification of a test subject level with specific control levels can be found, for example, at claim 12 as originally filed ("*d) determining whether the level of said one or more gene transcripts of step a) classify with the levels of said transcripts in step b) as compared with the levels of said transcripts in step c)*"), at paragraph [0136] (relating to "*Methods that can be used for class prediction analysis*"), [0398] ("*Blood samples were taken from patients who were diagnosed with osteoarthritis and a specific stage of osteoarthritis as defined herein. Gene expression profiles were then analysed and compared to profiles from patients unaffected by any disease.*") and paragraph [0400], describing the osteoarthritis versus healthy control clustering data shown in Figure 22.

Thus, new claim 58 requires, for classifying gene expression in the test subject relative to the controls, a clearly restricted comparison between test subject and control subject RNA levels, and a determination that the RNA level in the test subject is statistically similar to levels in either control subjects having osteoarthritis or to healthy control subjects, while conversely being statistically different from levels in healthy

control subjects or in control subjects having osteoarthritis, respectively. Thus, according to the claim the gene expression of the test subject is classified with that of one of the two types of control subjects with which it is statistically similar, where it is statistically different from that of the other type of control subjects.

Applicant submits that since they are not drawn to a method of "diagnosis", but rather are merely drawn to a method of classifying gene expression in a test subject relative to controls, the instant claims address the Examiner's remarks whereby the specification teaches that NTN4 is differently expressed in individuals with a disease other than osteoarthritis versus healthy controls, and is not differently expressed versus healthy controls in subjects having osteoarthritis which are subject to drug treatment, have a disease other than osteoarthritis or have particular stages of osteoarthritis. In the interest of clarifying that the controls may exclude the aforementioned types of controls, claim 59 depending from claim 58 has been added, limiting the control subjects to those which are not subject to systemic steroids and which do not have rheumatoid arthritis, hypertension, obesity, allergies, mild osteoarthritis or severe osteoarthritis. In the interest of clarifying that the control subjects having osteoarthritis may further have moderate osteoarthritis or marked osteoarthritis, new claims 60 and 61 have been added, limiting the control subjects having osteoarthritis to those which have moderate osteoarthritis or marked osteoarthritis, respectively.

Thus, Applicant submits that the instant claims address the Examiner's concerns by reciting a method for merely classifying gene expression in a test subject relative to that in narrowly defined control subjects which are either healthy or have osteoarthritis, and where determination of a narrowly defined relationship in gene expression levels logically classifies gene expression in the test subject relative to either type of the control subjects, in accordance with the teachings of the specification, for example, at Example 24 as pointed out by the Examiner at p. 7 of the Office Action dated April 2, 2007, and as described above.

Level and Magnitude of Differential Expression

Applicant submits that since they are not drawn to a method of "diagnosis", but are rather merely drawn to a method of classifying gene expression in a test subject relative to control subjects, the instant claims render moot the Examiner's concerns whereby the claims do not set forth the directionality and magnitude of the difference in NTN4 RNA levels in blood samples between a test subject and control subjects. The fact that the instant claims merely require determination of a statistical similarity or a statistical difference in gene expression levels between the test subject and the control subjects clearly makes it unnecessary to include the direction and magnitude of differential gene expression for enablement.

Applicant submits that the instant claims are enabled since the specification teaches that the differential expression NTN4 between subjects having osteoarthritis and healthy subjects is statistically significant, as evidenced by its P-value of <.05 as indicated at Figure 22 of the specification. The dendrogram of Figure 22 demonstrates that the NTN4 gene is one of a number of genes which demonstrate a statistically significant difference as between a population of subjects having osteoarthritis and healthy (normal) subjects. Therefore, Applicant has taught that there is a significant difference in differential expression of NTN4 between subjects having osteoarthritis and healthy subjects, and further has taught to compare the level of expression of NTN4 in a test subject with subjects having osteoarthritis and healthy subjects, using classification methods to determine the similarity or difference in gene expression levels therebetween. Support for such teachings can be found in the published application, for example, as described above, at paragraph [0128] ("*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"), at paragraph [0129] ("*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"), at claim 12 as originally filed ("*d) determining whether the level of said one*

or more gene transcripts of step a) classify with the levels of said transcripts in step b) as compared with the levels of said transcripts in step c)"), at paragraph [0136] (relating to "*Methods that can be used for class prediction analysis*"), [0398] ("*Blood samples were taken from patients who were diagnosed with osteoarthritis and a specific stage of osteoarthritis as defined herein. Gene expression profiles were then analysed and compared to profiles from patients unaffected by any disease.*") and at paragraph [0400], describing the osteoarthritis versus healthy control clustering data shown in Figure 22.

Predictability

Applicant submits that since they are not drawn to a method of "diagnosis", but are rather merely drawn to a method of classifying gene expression in a test subject relative to control subjects, the instant claims render moot the Examiner's concerns whereby the method of the claims is unpredictable. The fact that the instant claims merely require determination of a statistical similarity and a statistical difference in gene expression levels between the test subject and the control subjects, where the difference in gene expression between the control subjects is statistically significant, as taught by the specification, as described above, clearly renders the method of the instant claims sufficiently predictable for enablement.

The Applicant would point out that the results disclosed by Cheung *et al.* cannot be reliably extrapolated to primary blood samples since the lymphoblastoid cells employed by Cheung *et al.* are significantly modified relative to primary blood cells, due to being cultured cell lines generated by immortalization of primary human cells derived from "CEPH" families, as indicated in Reference no. 10 of Cheung *et al.* (Dausset *et al.*, 1990. Genomics 6:575; enclosed) at p. 575, right column, 1st paragraph. Applicant wishes to point out that immortalized cultured cell lines such as the lymphoblastoid cells taught by Cheung *et al.* undergo significant genetic modification such as strong genome-wide demethylation (refer, for example, to enclosed abstract of: Vilain *et al.*, 2003. DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogenet Cell Genet. 90:93), as a result of extensive *in-vitro* culturing in the absence of immune or apoptotic mechanisms which function to eliminate mutated cells in the body. As such,

immortalized CEPH lymphoblastoid cells may represent a particularly unsuitable cell type for modeling gene expression variability in primary blood cells.

Applicant would also respectfully disagree with the contention in Wu et al. that expression data needs to be interpreted in view of other biological knowledge. Differential gene expression which is reproducible, and is correlated with the state of health or disease of the individual does not necessarily result directly from the state of disease of the individual. Rather these changes in expression can be as a result of a downstream effect of pathogenic processes, and it is not necessary that the biological relevance of the data be known to allow this difference in expression to be useful as a biomarker. For example prostate-specific phosphatase and prostate-specific antigen (PSA) were long used as biomarkers without an understanding of their function (refer, for example, to the enclosed abstracts of: Chu TM, 1990, (Prostate cancer-associated markers. Immunol. Ser. 53:339-56; and Diamandis EP., 2000. Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 46:896-900).

In light of the amendments and above remarks, the Applicant contends that the claims are fully enabled, and respectfully requests reconsideration and withdrawal of the instant rejections.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. No new matter is added. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

Date: October 2, 2007

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Encl.

Abstract of: Casey et al., 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9;

Abstract of: Chu TM, Prostate Cancer-Associated Markers (1990) Immunol. Ser. 53:339-56;

Abstract of: Diamandis EP Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 2000 Jul: 46(7) 896:900; and

Abstract of: Vilain A., Bernardino J., Gerbault-Seureau M., Vogt N., Niveleau A., Lefrancois D., Malfoy B., and Dutrillaux B. (2000) DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogen. Cell Genet. (2000) 90 (1-2) 93-101.

Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds., Fig. A.23;

A

Abstract of: Casey et al., 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9

Routine fixation and paraffin embedding destroys many hematopoietic and lymphoid differentiation antigens detected by flow cytometry or frozen section immunohistochemistry. On the other hand, morphologic evaluation is difficult in flow cytometric or frozen section studies. A simplified three-step plastic embedding system using acetone-fixed tissues embedded in glycol-methacrylate (GMA) resin has been found to provide both excellent morphologic and antigenic preservation. With our system, a wide variety of antigens are detected in plastic sections without trypsinization or prolonged embedding procedures; **pan-B** (**CD19**, CD22), **pan-T** (CD7, CD5, **CD3**, CD2), T-subset (CD4, CD8, CD1, CD25) markers as well as surface immunoglobulin and markers for myeloid and mononuclear-phagocyte cells are preserved. In summary, modifications of plastic embedding techniques used in this study simplify the procedure, apparently achieve excellent antigenic preservation, and facilitate evaluation of morphologic details in relation to immunocytochemical markers.

B

Abstract of: Chu TM, Prostate Cancer-Associated Markers (1990) Immunol. Ser. 53:339-56.

Immunodiagnosis of prostate cancer is at a more advanced stage than that of most other tumors. Two well-known markers, prostatic acid phosphatase and prostate-specific antigen, have been used in the clinical management of patients. Prostate-specific antigen is a more sensitive and reliable marker than prostatic acid phosphatase. Serum prostate-specific antigen is effective in monitoring disease status, predicting recurrence, and detecting residual disease. Prostate-specific antigen is a tool for the histological differential diagnosis of metastatic carcinomas, especially in the identification of metastatic prostate tumor cells in distant organs and in the differentiation of primary prostate carcinoma from poorly differentiated transitional cell carcinoma of the bladder. Few data on biological function are available. Prostatic acid phosphatase functions as a phosphotyrosyl-protein phosphatase and prostate-specific antigen as a protease. Physiological function in the prostate remains to be elucidated. Several of the prostate-specific and prostate-tumor-associated antigens, as well as a putative prostate tumor-specific antigen, as recognized by monoclonal antibodies are available. Clinical evaluationn of these potential markers is not yet available.

C

Abstract of: Diamandis EP Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 2000 Jul: 46(7) 896:900

BACKGROUND: Prostate-specific antigen (PSA) is a valuable prostatic cancer biomarker that is now widely used for population screening, diagnosis, and monitoring of patients with prostate cancer. Despite the voluminous literature on this biomarker, relatively few reports have addressed the issue of its physiological function and its connection to the pathogenesis and progression of prostate and other cancers. APPROACH: I here review literature dealing with PSA physiology and pathobiology and discuss reports that either suggest that PSA is a beneficial molecule with tumor suppressor activity or that PSA has deleterious effects in prostate, breast, and possibly other cancers. CONTENT: The present scientific literature on PSA physiology and pathobiology is confusing. A group of reports have suggested that PSA may act as a tumor suppressor, a negative regulator of cell growth, and an apoptotic molecule, whereas others suggest that PSA may, through its chymotrypsin-like activity, promote tumor progression and metastasis. SUMMARY: The physiological function of PSA is still not well understood. Because PSA is just one member of the human kallikrein gene family, it is possible that its biological functions are related to the activity of other related kallikreins. Only when the physiological functions of PSA and other kallikreins are elucidated will we be able to explain the currently apparently conflicting experimental data.

D

Abstract of: Vilain A., Bernardino J., Gerbault-Seureau M., Vogt N., Niveleau A., Lefrancois D., Malfoy B., and Dutrillaux B. (2000) DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogen. Cell Genet. (2000) 90 (1-2) 93-101

In order to gain more insight into the relationships between DNA methylation and genome stability, chromosomal and molecular evolutions of four Epstein-Barr virus-transformed human lymphoblastoid cell lines were followed in culture for more than 2 yr. The four cell lines underwent early, strong overall demethylation of the genome. The classical satellite-rich, heterochromatic,juxtacentromeric regions of chromosomes 1, 9, and 16 and the distal part of the long arm of the Y chromosome displayed specific behavior with time in culture. In two cell lines, they underwent a strong demethylation, involving successively chromosomes Y, 9, 16, and 1, whereas in the two other cell lines, they remained heavily methylated. For classical satellite 2-rich heterochromatic regions of chromosomes 1 and 16, a direct relationship could be established between their demethylation, their undercondensation at metaphase, and their involvement in non-clonal rearrangements. Unstable sites distributed along the whole chromosomes were found only when the heterochromatic regions of chromosomes 1 and 16 were unstable. The classical satellite 3-rich heterochromatic region of chromosomes 9 and Y, despite their strong demethylation, remained condensed and stable. Genome demethylation and chromosome instability could not be related to variations in mRNA amounts of the DNA methyltransferases DNMT1, DNMT3A, and DNMT3B and DNA demethylase. These data suggest that the influence of DNA demethylation on chromosome stability is modulated by a sequence-specific chromatin structure. Copyright 2000 S. Karger AG, Basel.

E

Short Contents | Full Contents Other books @ NCBI

Navigation

About this book

Appendix I. Immunologists' Toolbox

Immunization.

The detection, measurement, and characterization of antibodies and their use as research and diagnostic tools.

Isolation of lymphocytes.

Characterization of lymphocyte specificity, frequency, and function.

Detection of immunity *in vivo*.

Manipulation of the immune system.

Search

Go

This book All books

PubMed

Immunobiology ➔ **Appendix I. Immunologists' Toolbox** ➔ Isolation of lymphocytes.

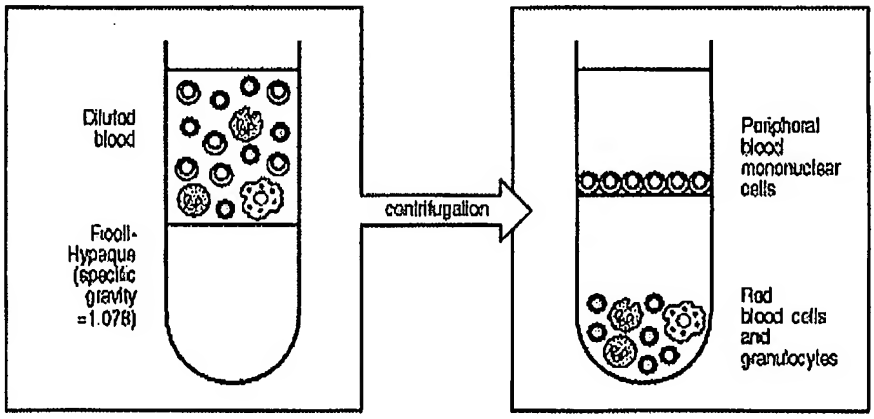

Figure A.23. Peripheral blood mononuclear cells can be isolated from whole blood by Ficoll-Hypaque™ centrifugation. Diluted anticoagulated blood (left panel) is layered over Ficoll-Hypaque™ and centrifuged. Red blood cells and polymorphonuclear leukocytes or granulocytes are more dense and centrifuge through the Ficoll-Hypaque™, while mononuclear cells consisting of lymphocytes together with some monocytes band over it and can be recovered at the interface (right panel).